

Amendments to the Specification:

On page 8, please replace the paragraph at lines 23-30 with the following replacement paragraph:

-- The coat protein is selected as the site for cleavage on the grounds that it is available to cleaving agents in the host cells harbouring the virus or at the surface of the virus itself. Thus, in a preferred embodiment, virus preparations may be treated with a cleaving agent, in order to render virions having cleavable coat proteins unable to mediate infection of host cells.

Alternatively, cells infected with the virus may be treated with a cleaving agent active within the cell, which will prevent packaging of virus comprising a cleavable coat protein. As used herein, the terms pIII, p3, g3 and gene III are equivalent alternative abbreviations for bacteriophage coat protein III. --

Please replace the paragraph at page 9, from lines 22-26, with the following replacement paragraph:

-- If the protease cleavage site incorporated in the coat protein remains uncleaved, therefore, the virus is capable of assembly into functional virions and retains the potential to infect host cells. If the protease cleavage site is cleaved, however, the structure of the viral coat protein will be compromised and the viral virus will lose at least part of its potential to infect host cells.--

Please replace the paragraph at page 12, line 30 to page 13, line 8 with the following replacement paragraph:

-- A second configuration of the present invention concerns the use of tags to allow isolation of correctly folded heterologous polypeptides, exploiting the ability of correctly folded polypeptides to protect a cleavable site on ~~to~~ or near to an associated tag; The insertion of a polypeptide between the stable tag fused to the N-terminus of the viral coat protein and the coat protein itself, followed by cleavage, provides a means of selection for virus bearing proteins that are resistant to proteolysis and are folded. Thus only virions, whose inserted polypeptide is not

degraded, will keep the tag fusion as part of their coat, and only these virions can therefore be captured by affinity purification using this tag. After elution the affinity captured phases from the ligand, these phages can be propagated and subjected to further rounds of the same selection procedure. --

Please replace the paragraph at page 12, line 30 to page 13, line 8 with the following replacement paragraph:

--Phage is incubated under a range of denaturing conditions in vitro and then restored to native conditions immediately before infection of bacteria. The incubation of phage in 10 M urea, or extremes of pH (as low as pH 2, and as high as pH 12) and temperature (as high as 60°C) did not lead to a major loss of infectivity (Table 1). This indicates that the phage is either resistant to denaturing conditions or that if it does unfold it is able to refold rapidly. However with GndHCl Guanidine hydrochloride (GndHCl) a 5 fold loss in phage infectivity is observed above 5 M and a further 5 fold loss at 8 M (Table 1). --

Please replace Table 1 on page 17 with the following replacement table:

-- **Table 1.** Stability of wild type fd-DOG under different conditions. The infectivity (TU/ml  $\times 10^{10}$ ) was measured (see Materials and Methods) and has an estimated error of about  $\pm 6\%$ .

Urea (60°C, 90 min)	Control	2 M	4 M	6 M	8 M	12.0
	0.56	0.64	0.32	0.32	0.80	0.68
GndHC1 (37°C, 90 min)	Control	2 M	4 M	5 M	6 M	7 M
	0.72	0.60	0.70	0.16	0.13	0.16
pH (37°C, 30 min)	Control	pH 2.2	pH 4.0	pH 7.4	pH 10	pH 12
	1.5	0.46	1.3	1.5	1.4	0.40
Temperature (30 min)	Control	22°C	37°C	60°C		
	9.7	8.3	9.6	12.0		
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Please replace the paragraph at page 25, lines 5-10 with the following replacement paragraph:

-- All restriction enzymes, T4 ligase are obtained from New England Biolabs. Taq DNA polymerase is obtained from HT Biotechnology. Pfu DNA polymerase is obtained from Stratagene. Ultrapure dNTP from Pharmacia. Proteases and the protease inhibitor ~~Pefabloc~~ PEFABLOC<sup>TM</sup> are obtained from Boehringer Mannheim, except chymotrypsin and trypsin TPCK-treated which are obtained from Sigma. All other chemical are likewise obtained from Sigma. --

Please replace the paragraph at page 25, lines 21-27 with the following replacement paragraph:

-- The phage vector fd-DOG [43] is used as parent vector for construction of the protease cleavable fd-K108. Unique restriction sites (SfiI, KpnI) are introduced into the glycine rich spacer region between D2 and D3 using the ~~Sculptor~~ SCULPTOR<sup>TM</sup> *in vitro* mutagenesis system (Amersham) and the oligonucleotide pklinker (Table 4). Further restriction sites (ApaI, SalI) and sequence encoding a protease cleavage site are cloned between the SfiI and KpnI sites using the oligonucleotides polyXafor and polyXaback to create the vector fd- K108. --

Please replace the paragraph at page 26, lines 1-14 with the following replacement paragraph:

-- A protease cleavable phagemid vector is derived from fd-K108 much as above except using pCANTAB 3 (Pharmacia). A FLAG-tag is introduced at the N-terminus of D1 by cloning of a NotI-SfiI fragment generated by PCR and primers Flagprimer and LSPAback. To circumvent deletions due to repeated sequence in the D2-D3 linker, the codon usage of the polylinker region is changed in two steps (a) using a Bam-SfiI fragment generated by PCR and primers RECGLYfor and LIBSEQfor, screening recombinants by PCR and the primers LSPAfor and LSPAback, (b) using a KpnI-ClaI fragment generated by PCR and the primers RECGLYback and LIBSEQback, screening recombinants using LSPAfor and LSPAback. The resulting vector

is pK1. The entire p3 gene is sequenced using PCR cycle sequencing with fluorescent dideoxy chain terminators (Applied Biosystems) [45]. The "out of frame" vector pK2 is derived from pK1 by site direct mutagenesis using the oligo delCKpn and the Sculptor SCULPTOR™ in vitro mutagenesis system (Amersham) Amersham kit. The precise sequences of pK1 and pK2 are set forth in Kristensen et al., (1998) *Folding & Design* 3: 321-328. --

Please replace the paragraph at page 27, lines 23-26 with the following replacement paragraph:

-- For resistance to proteases in the presence of denaturants samples are prepared as above for urea and temperature denaturation. To 90  $\mu$ l aliquots 10  $\mu$ l trypsin (1 mg/ml) is added, after 5 min at room temperature 4  $\mu$ l Pefabloc PEFABLOC™ protease inhibitor (100 mM) is added and the samples are infected into TG1 as above. --

Please replace the paragraph at page 28, lines 18-26 with the following replacement paragraph:

-- At each temperature approximately  $10^{10}$  phage displaying the barnase mutants or villin (ampicillin resistant) is mixed with a cleavable control fd-K108 (tetracycline resistant), and a non-cleavable control phagemid, a chloramphenicol resistant derivative of pHEN1, rescued with KM 13 in a total volume of 90/ $\mu$ l of buffer A. After equilibration for 20-30 min at the temperature indicated, 10  $\mu$ u trypsin (5  $\mu$ g/ml) is added and the incubation continued for 2 min. Trypsin is neutralised by adding 4  $\mu$ l 100 mM Pefabloc PEFABLOC™ protease inhibitor. Infection and serial dilution is performed in TG-1 as above and aliquots are plated on TYE plates containing 100,  $\mu$ g/ml ampicillin + 1 % glucose, 30,  $\mu$ g/ml chloramphenicol + 1 % glucose or 15  $\mu$ g/ml tetracycline. --

Please replace the paragraph at page 28, line 30 to page 29, line 5 with the following replacement paragraph:

-- 10  $\mu$ l of serial dilutions of the barnase mutant phage A is mixed with 10  $\mu$ l of the non- diluted barnase mutant phage B in 70  $\mu$ l buffer A. After 30 min incubation at 20°C or 37°C 10  $\mu$ l trypsin

(5,  $\mu\text{g}/\text{ml}$ ) is added. Following 2 min. of digestion 4  $\mu\text{l}$  Pefabloc PEFABLOC<sup>TM</sup> protease inhibitor (100 mM) is added. The phage are infected into TG1 as above. A second round of selection are performed by scraping bacteria in 3 ml 2xTY, 50,  $\mu\text{l}$  inoculated into 50 ml 2xTY/Amp/Glu and the phagemid rescued and phage prepared as above. Clones are analysed by PCR using the primers LSPAfor and LSPAback followed by restriction digestion using DdeI. --

Please replace the paragraph at page 32, lines 14-20 with the following replacement paragraph:

-- Expression of polymerase in the supernatant is made by infection of E. coli HB2151 with selected phagemids [25,27] except that the IPTG concentration is 0.1 mM instead of 1 mM. About 10  $\mu\text{l}$  of supernatant is loaded on a polyacrylamide gel for electrophoresis (Novex); the gel is blotted onto nitrocellulose (Protran, Schleicher and Schuell) and an anti-Taq polymerase antibody (Taqstart, Clontech), and a goat anti- mouse IgG-horseradish peroxidase (Sigma) prior to detection on autoradiography films by chemiluminescence ( ECL ECL<sup>TM</sup> chemiluminescence reagents, Amersham). --

Please replace the paragraph at page 37, lines 15-25 with the following replacement paragraph:

-- The fusion protein is cloned on a phagemid (pHEN1) [25], and is rescued by a helper phage. The polymerase fragments are shown to be displayed on the phage (after rescue with helper phage) by binding of the phage to wells coated with anti-polymerase antibodies as detected by ELISA (not shown). The phage are also analysed by Western blot using anti-p3 or anti-polymerase antibodies. This confirms the presence of the fusion protein, but also indicates contamination by free polymerase. Presumably this arises by secretion from the bacterial host through incomplete suppression of the amber stop codon or by cleavage from the phage surface. This is removed by a further step of ultracentrifugation or by size exclusion chromatography. The purified phages are assayed for DNA polymerase activity in a primer/template extension assay with radioactively labelled  $\alpha^{32}\text{P-dCTP}$   $\alpha^{32}\text{P-dCTP}$  and found to be active. --

Please replace page 39 with replacement page 39 enclosed herein.

Please replace the paragraph at page 40, lines 1-6 with the following replacement paragraph:

-- within the insert) create an open reading frame spanning barnase, the insert itself and p3 and result in infectious phage particles containing p3 in the phage coat. In these recombinant clones barnase is followed by the insert, which is then followed by the amino acid residues AGGAAA (SEQ ID NO: 80) before the start of the p3 protein. This AGGAAA (SEQ ID NO: 80) peptide should provide enough flexibility in the fusion protein to enable the infectivity function of p3 and the access of the protease to the N-terminal appendices of p3. --